The Journal of Experimental Biology 211, 3504-3511
Published by The Company of Biologists 2008
doi:10.1242/jeb.017848

Simultaneous color contrast in the foraging swallowtail butterfly, *Papilio xuthus*

Michiyo Kinoshita[1,*], Yuki Takahashi[2] and Kentaro Arikawa[1]

[1]Laboratory of Neuroethology, The Graduate University for Advanced Studies (Sokendai), Shonan Village, Hayama 240-0193, Japan and [2]Graduate School of Integrated Science, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

*Author for correspondence (e-mail: kinoshita_michiyo@soken.ac.jp)

Accepted 15 September 2008

SUMMARY

This study demonstrates that the color vision of foraging Japanese yellow swallowtail butterflies, *Papilio xuthus*, involves simultaneous color contrast. We trained newly emerged *Papilio* to select a disk of pale green among a set of differently colored disks presented on a black background. When the same set of disks was presented on blue background, the pale green-trained butterflies selected blue-green. The difference in spectra between pale green and blue green was similar to the spectrum of yellow for human vision, suggesting that blue induces yellow. Similarly, the pale green-trained *Papilio* selected a more bluish spring green on yellow background. We also trained *Papilio* with orange disks and tested on a green and violet background. The results showed that green induced violet and *vice versa.* Taken together, we concluded that simultaneous color contrast of *Papilio* is similar to the effect of complementary colors in human color vision.

Key words: insect, visual system, complementary color, Lepidoptera, color constancy.

INTRODUCTION

Perception of a color is affected by the color of its surrounding area or background. This phenomenon, called simultaneous color contrast, is one of the most important properties of color vision. A gray area appears yellowish for humans, for example, when it is surrounded by blue; in other words, the surrounding blue induces yellow on the gray area. Conversely, a yellow background induces blue. Such pairs of colors are called complementary and produce white when mixed.

Simultaneous color contrast is thought to be one of several elementary processes underlying color constancy (Hurlbert and Wolf, 2004; Vanleeuwen et al., 2007), the phenomenon in which the color of an object is constant under various illumination colors. Both color constancy and color contrast require the neuronal process of integrating spatial chromatic information, as was shown in behavioral experiments in goldfish (Dörr and Neumeyer, 1997; Neumeyer et al., 2002). In goldfish, the horizontal cells in the outer retina provide inhibitory feedback to the cone photoreceptor cells and integrate chromatic and spatial information (Kamermans et al., 1998). Such a mechanism is one of the most plausible explanations for the physiological basis of color constancy and color contrast.

Color contrast is also known in an insect, the honeybee *Apis mellifera*. Honeybees have a trichromatic system based on the ultraviolet (UV)-, blue (B)- and green (G)-sensitive photoreceptors in the retina (Frisch, 1914; Menzel and Backhaus, 1989). Neumeyer (Neumeyer, 1980) trained foraging honeybees to select a blue-green disk from nine disks of different colors placed on a gray background and tested their color preference on blue as well as on yellow backgrounds. The results demonstrated that the blue background shifted their preference toward a shorter wavelength, whereas the yellow background shifted preference toward a longer wavelength (Neumeyer, 1980).

Recent progress in the study of insect color vision has revealed that several lepidopteran species have color vision (Kelber and Pfaff, 1999; Kinoshita et al., 1999; Kelber et al., 2002; Zaccardi et al., 2006). The Japanese yellow swallowtail butterfly, *Papilio xuthus,* has been studied in detail in this respect, from the spectral organization of the retina (Arikawa, 2003) to behavioral evidence for color vision (Kinoshita et al., 1999). The retina of *Papilio xuthus* contains at least six classes of spectral receptors: the UV, violet (V), B, G, red (R), and broadband (BB) receptors. Its color vision is probably tetrachromatic, however, based on the UV, B, G and R receptors (Koshitaka et al., 2008). *Papilio xuthus* has even been shown to have color constancy (Kinoshita and Arikawa, 2000).

To understand the neuronal mechanism underlying color constancy in *Papilio*, it is important to know whether and how the elementary process of simultaneous color contrast works. In this study, we first tested whether *Papilio* is capable of simultaneous color contrast. We trained a newly emerged *Papilio* to select a pale green or orange disk from among disks of other colors presented on a black background. Then we let the butterfly select colors on both gray and some colored backgrounds. Although they correctly selected the training color on the gray background, their selection was systematically affected by background colors, indicating that *Papilio* is indeed capable of simultaneous color contrast.

MATERIALS AND METHODS

Animals

We used laboratory-raised spring-form females of the Japanese yellow swallowtail butterfly, *Papilio xuthus* L. We first collected females in the field around the campus and let them lay eggs on citrus plants in the laboratory. Hatched larvae were fed fresh citrus leaves at 25–27°C under a light regime of 10 h:14 h L:D, which induces pupal diapause. The diapausing pupae were chill-treated at 4°C for at least 3 months, after which they were allowed to emerge at 25°C. The day of emergence was defined as post-emergence day 1. Each newly emerged butterfly was numbered and put into a white

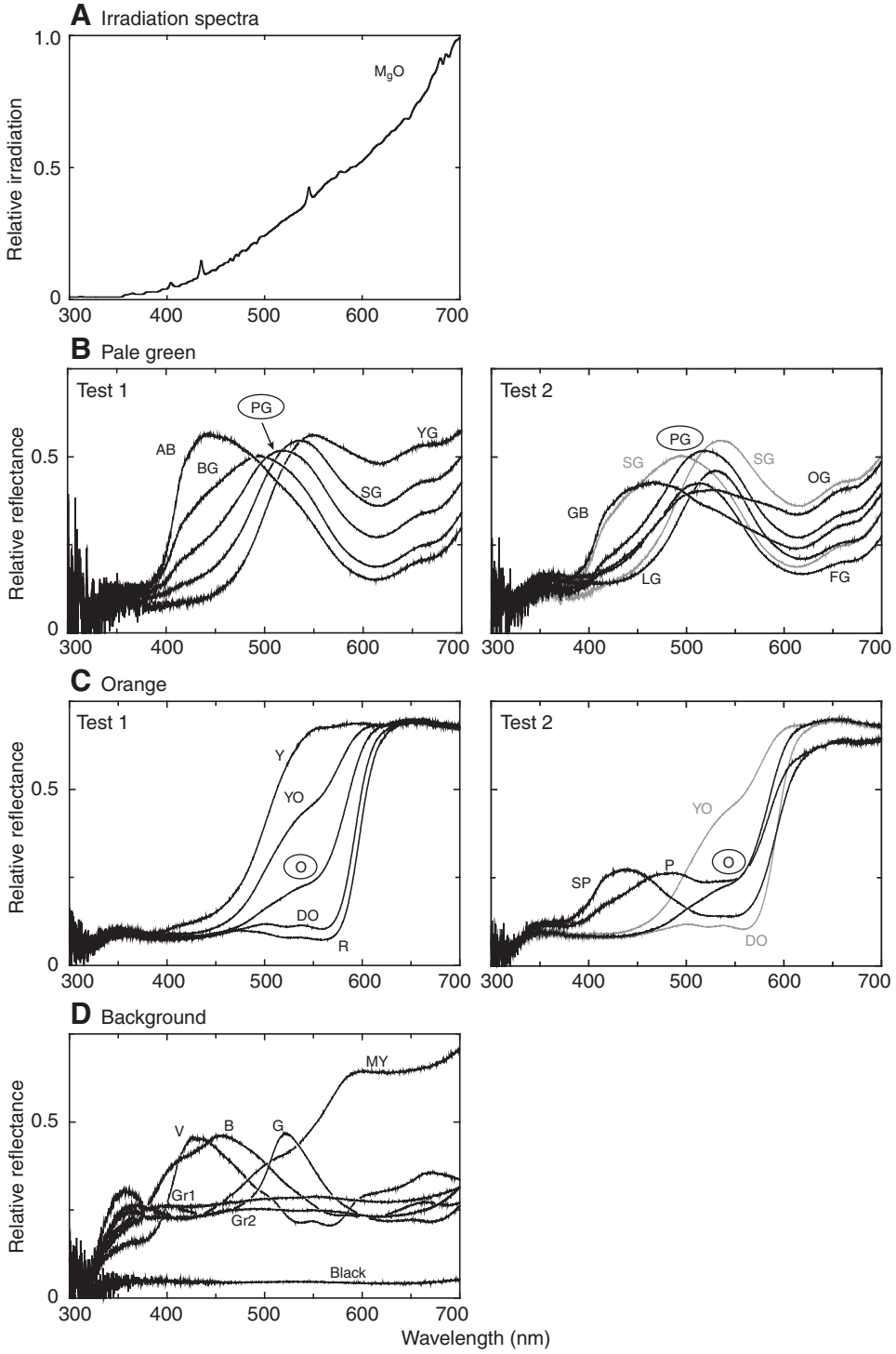

Fig. 1. Illumination and stimuli. (A) Irradiation spectrum of illumination measured as the reflection of MgO-coated surface. (B) Reflectance spectra of colored papers used for the experiments with pale-green (PG)-trained butterflies. (C) Reflectance spectra of colored papers used for the experiments with orange (O)-trained butterflies. (D) Reflectance spectra of background cardboards.

styrofoam box covered with gauze. These butterflies were fed with sucrose solution only when they were trained (see below).

Illumination and stimuli

Behavioral experiments were carried out in a cage (80 cm×60 cm×45 cm) set in a room at 30±1°C with relative humidity kept higher than 50%. The cage was illuminated with fluorescent tubes and halogen bulbs, making the luminance at the floor of the cage ~3000 lx. The illumination contained very little UV light (Fig. 1A). Because color vision of foraging *Papilio* is assumed to be tetrachromatic based on the UV, B, G and R receptors (Koshitaka et al., 2008), this illumination was not 'white' for butterflies. Even a very small amount of UV light could provide substantial input to the UV receptor system, but we previously demonstrated color vision (Kinoshita et al., 1999) as well as color constancy (Kinoshita and Arikawa, 2000) in *Papilio* under the same illumination conditions, so this illumination should be sufficient to demonstrate whether or not simultaneous color contrast also exists in *Papilio*.

For the visual stimuli, we prepared paper disks of sixteen different colors using an inkjet printer (PM800C, EPSON Japan) and cardboard of four colors as background that were selected based on a series of pilot experiments (Fig. 1). For clarity, we hereafter

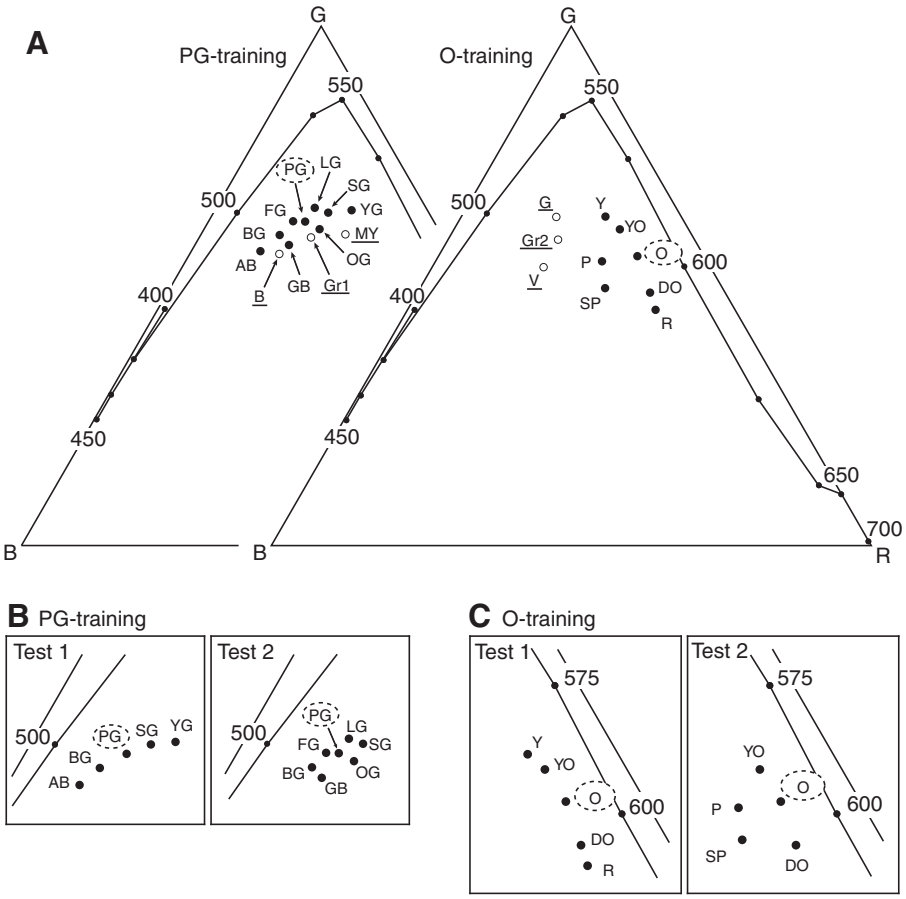

Fig. 2. Color loci of color disks and background cardboards on a presumptive three-dimensional color space of *Papilio xuthus*. Thin lines with numbers show the loci of monochromatic lights. (A) Color loci of two series of color disks and backgrounds, each relating to the training colors; circled letters PG (pale green) and O (orange). Filled circles in the left triangle are colors of disks used for PG training: AB, aqua blue; BG, blue green; FG, forest green; LG, lime green; SG, spring green; YG, yellow green; OG, olive green; GB, grayish blue. Open circles in the left triangle are colors of the background for PG training: B; blue; G, green; MY, mustard yellow; Gr1, gray 1. Filled circles in the right triangle are colors of disks used for O training: Y, yellow; YO, yellow orange; DO, dark orange; R, red; SP, salmon pink; P, pink. Open circles in the left triangle were colors of the background for O training: G, green; V, violet; Gr2, gray 2. (B) Enlarged view of color loci of colors presented with PG for tests 1 and 2. Loci of colors in test 1 are arranged approximately in linear fashion, whereas those in test 2 are distributed around the training color, PG. (C) Enlarged view of color loci of colors presented with O for tests 1 and 2.

refer to all these colored papers using the name of the color as perceived by humans.

We used pale green (PG) and orange (O) as training colors. We prepared two series of color disks, each relating to these two training colors. The reflectance spectra are shown in Fig. 1B–D. The first set consisted of pale green (PG, training color), aqua blue (AB), blue green (BG), spring green (SG), yellow green (YG), forest green (FG), grayish blue (GB), olive green (OG) and lime green (LG) (Fig. 1B). The second set consisted of orange (O, training color), yellow (Y), yellowish orange (YO), dark orange (DO), red (R), salmon pink (SP) and pink (P) (Fig. 1C).

Background colors were black (Bl), two different shades of gray (Gr1 and Gr2), blue (B), green (G), mustard yellow (MY) and violet (V) (Fig. 1D). Gr1, B and MY were used for testing PG-trained butterflies, whereas Gr2, G and V were used for testing O-trained butterflies. Reflectance spectra of all stimuli were measured in the wavelength region of 300–700 nm by a spectrophotometer (HR2000, Ocean Optics, Inc., USA) using the peak of the reflection of the MgO-coated surface as 1.0.

We calculated the color loci of all colors including those for background in the presumptive *Papilio* color space based on the B, G and R receptors as follows. We first calculated:

$$X = \int_{300}^{700} 1.00 \cdot S_{\mathrm{b}}(\lambda) I(\lambda) R_{\mathrm{i}}(\lambda) d\lambda$$
$$Y = \int_{300}^{700} 0.72 \cdot S_{\mathrm{g}}(\lambda) I(\lambda) R_{\mathrm{i}}(\lambda) d\lambda \ , \qquad (1)$$
$$Z = \int_{300}^{700} 0.17 \cdot S_{\mathrm{r}}(\lambda) I(\lambda) R_{\mathrm{i}}(\lambda) d\lambda$$

where λ is the wavelength; $I(\lambda)$ is the irradiation spectrum of illumination (Fig. 1D); $R_{\mathrm{i}}(\lambda)$ is the reflectance spectrum of the colored paper i (Fig. 1B–D); and $S_{\mathrm{b}}(\lambda)$, $S_{\mathrm{g}}(\lambda)$ and $S_{\mathrm{r}}(\lambda)$ are the (normalized) spectral sensitivities of the B, G and R receptors, respectively, determined by intracellular recording. The numbers indicate relative sensitivities calculated from the values at 460 nm (B), 540 nm (G) and 600 nm (R) in the action spectrum of foraging behavior (Koshitaka et al., 2004) (Fig. 4C). We obtained the coordinates for a two-dimensional plot of the color triangle taking $x=X/(X+Y+Z)$, $y=Y/(X+Y+Z)$ and $z=Z/(X+Y+Z)$ (Fig. 2).

The visual stimuli were arranged in three patterns: one disk, five disks and seven disks (Fig. 3A–C). The disks (diameter 4.6–7 cm) were mounted on background cardboard (size 55 cm×35 cm) of a particular color. The entire pattern was covered with anti-reflective glass when presented to the butterfly.

For a behavioral demonstration of simultaneous color contrast, we selected the color disks in two ways, hereafter called test 1 and test 2. Test 1 was designed to investigate how far the locus of the selected color moves in the color space as a consequence of the colored background. We selected five colors whose loci are arranged more or less linearly in the color space: AB, BG, PG, SG and YG (Fig. 2B) for the PG-trained butterflies and Y, YO, O, DO and R (Fig. 2C) for the O-trained butterflies. These colors were presented as the five-disk pattern (Fig. 3B).

Test 2 was designed to investigate in which direction the locus of the selected color moves in the color space as a consequence of the colored background. We used FG, BG, GB, PG, OG, SG and LG (Fig. 2B) on the seven-disk pattern for the PG-trained butterflies and YO, SP, O, P and DO (Fig. 2C) on the five-disk pattern for the O-trained butterflies. The loci of these colors were

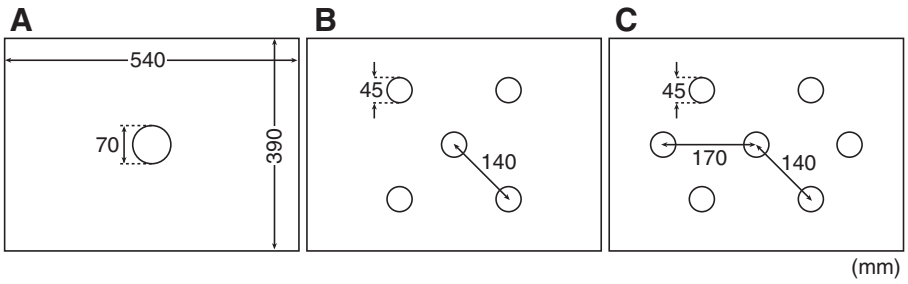

Fig. 3. Layout patterns of color disks. (A) Training pattern used from post-emergence day 2 to day 5 (1 disk). (B) Five-disk pattern. (C) Seven-disk pattern.

distributed radially with the locus of the training color in the center.

We calculated the *Papilio*-subjective brightness of paper *i*, B_i, by:

$$B_i = \int_{360}^{600} A(\lambda) I(\lambda) R_i(\lambda) d\lambda , \quad (2)$$

where $I(\lambda)$ is the irradiation spectrum (Fig. 1A), $A(\lambda)$ is the action spectrum of foraging behavior (Koshitaka et al., 2004) (Fig. 4C), and $R_i(\lambda)$ is the reflectance spectrum of the colored paper *i* (Fig. 1B–D). Because the range of the action spectrum was limited to 360–600 nm, we used this range for the calculation of brightness. Fig. 4 shows the relative subjective brightness of all color disks and backgrounds by taking the brightness of the black background (Bl) as 1.0. A gray background (Gr1 or Gr2) was selected so that the brightness was as close as possible to that of the training color, so we therefore used Gr1 for PG-trained experiments and Gr2 for O-trained experiments. Brighter grays than Gr1, which look whitish to humans, prevented *Papilio* from flying normally in the cage, and they frequently turned over on the floor.

Procedure of behavioral experiments

The behavioral experiments consisted of three sessions; training, control test and color contrast test.

In the training session, we trained butterflies to search for sucrose solution on a disk of a certain color among several other colored disks presented on a black background. The session was further divided into three steps. The first step was to feed a butterfly with 3% sucrose solution on a training pattern on post-emergence day 2. The pattern had a disk of either PG or O on a black background (Fig. 3A). As the second step we fed the butterfly similarly on day 3 but with 5% sucrose, since increasing the concentration was necessary to prolong the butterfly's life. We repeated the training with 5% sucrose for three more days. From post-emergence day 6, we started the third step, which was to present either five or seven disks for planned tests on a black background (Fig. 3B,C). We used the five-disk patterns for individuals prepared for test 1 of PG and O training and for test 2 of O training. For individuals prepared for test 2 of the PG training, we used the seven-disk pattern. On the disk of the training color, PG or O, among the five or seven disks on the pattern we fed the butterfly with 5% sucrose. We sometimes chased butterflies off the disk and let them visit the disk for ten times in 1 day. After every third visit, we randomly changed the relative position of color disks in order to prevent them from learning positions, but we did not see any sign of positional learning throughout the study. We repeated this training for three more days. The set of colors presented to a particular individual in the third step of training was also used in the following two sessions, the control test and the color contrast test.

The control test session started on post-emergence day 9 and was carried out before the butterfly was fed. In this test we checked whether or not the brightness of background could affect the visits of the trained butterflies. First we presented a disk of the training color and gave the butterfly about 10 µl of 10% sucrose on the disk to stimulate feeding motivation. Then we presented each butterfly with the same set of five or seven disks used in the third phase of training for the individual, but this time we used a gray background without providing sucrose on any disk. We defined the 'visit' as when the butterfly landed and touched a disk with its proboscis extended. We let the butterfly visit any disks five times and recorded the colors of the visited disks. After the third visit, we changed the relative position of the color disks. After the control test, we fed the butterfly on the disk of the training color in the five-disk or seven-disk pattern on a black background until it was satiated. If the butterfly did not visit any disks within 5 min after the test started, we stopped using that individual for the color contrast tests.

On post-emergence days 10 and 11, we carried out color contrast tests 1 and 2. In test 1, we presented selected colors that were linearly arranged in the color space on a gray or colored background (Fig. 2). We used different sets of individuals, both of which passed the control test on the gray background, on two different background colors (Figs 5 and 6). In test 2, we presented selected colors that were distributed around the locus of the training color (Fig. 2) on a gray or colored background. Here we could use the same sets of individuals, which passed the control test on gray background, on both colored backgrounds (Figs 5 and 6). We let the butterfly visit any disk five times and recorded the colors of the visited disks. We changed the relative position of the color disks after the third visit. If the butterflies did not visit any disk within 5 min after the test started, we stopped the experiment.

After the tests, we assessed whether the butterfly retained the motivation for selecting the training color or not. If it correctly visited the training color from the set of colors on a gray background, we concluded that it had kept the motivation and we accepted the results. If it did not, we could have rejected individual data from the analysis, but all individuals subjected to the final color contrast test were accepted. Individuals with deteriorated motivation were rejected in the previous two sessions.

In this study we also carried out multiple statistical tests. First we checked whether or not the trained butterflies randomly selected disks of different colors using the Kruskal–Wallis test. To identify which color was significantly selected among the colors presented in each test, we applied Tukey's HSD test. Finally, we used the χ^2 test to compare the overall pattern of selection in the control test with test results with colored backgrounds.

RESULTS

We used about 100 individuals for this study, 54 of which were successfully trained to either PG (26) or O (28). The rest were rejected during the training and the control test sessions or died in the course of the experiment. Most of the successful individuals acquired the ability to visit the disk of the training color by

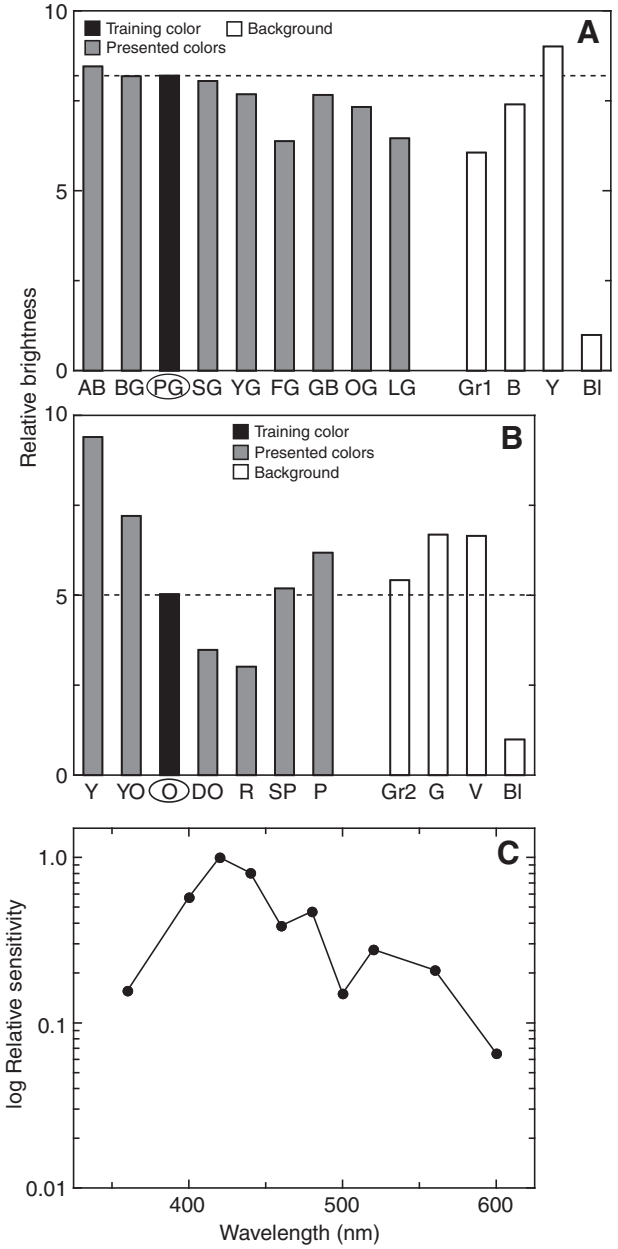

Fig. 4. Relative subjective brightness of all color disks and backgrounds, taking the brightness of the black background as 1.0. The dotted lines indicate the brightness of the training colors. (A) Colors related to the pale-green (PG) training. (B) Colors related to the orange (O) training. (C) Action spectrum of foraging behavior of freely flying *Papilio* [modified from Koshitaka et al. (Koshitaka et al., 2004)].

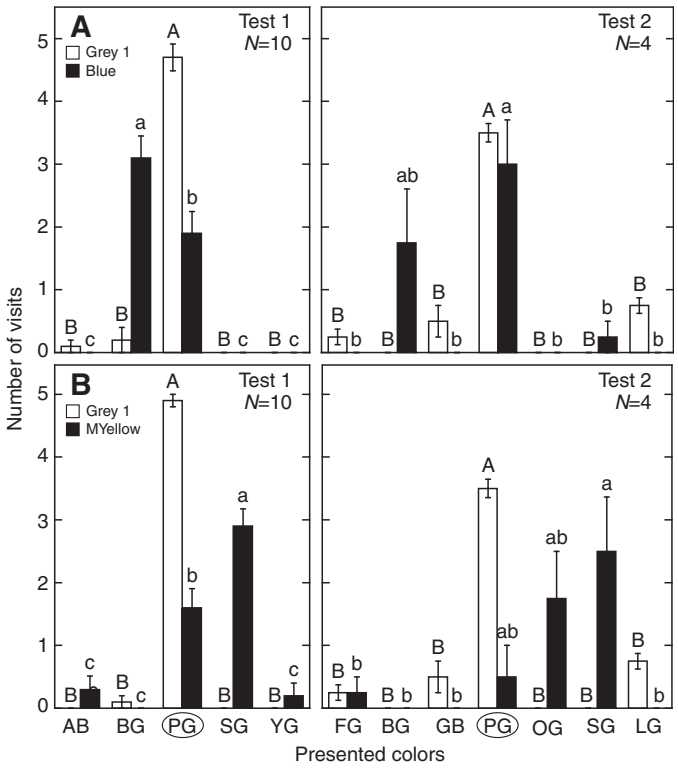

Fig. 5. Results of pale-green (PG)-trained *Papilio*. Colors in test 1 were arranged linearly in the color space, whereas colors in test 2 were arranged radially around PG, the training color (see Fig. 2B). (A) Effect of a blue (B) background. (B) Effect of a mustard yellow (MY) background. The choices were not random in each test. (A) Test 1: Gray (Gr1), H=42.57, P<0.0001; B, H=46.20, P<0.0001. Test 2: Gr1, H=19.46, P<0.01; B, H=20.66, P<0.001. (B) Test 1: Gr1, H=45.54, P<0.0001; MY, H=36.98, P<0.0001. Test 2: Gr1, H=19.46, P<0.01; MY, H=17.43, P<0.001; Kruskal–Wallis test). Each bar shows the mean (±s.e.m.) number of visits. Letters above bars indicate statistical significance. Bars with different letters are significantly different (Tukey's HSD test, P<0.05). Capital letters are used for the results of the control test, and lowercase letters are used for the result of the color contrast tests.

themselves in the first 3 days of training. On the final training day, all of them could select the correct disk from among the five or seven selected sets of color disks presented on a black background. In all the following tests, the choices of the trained butterflies were not random (P<0.05, Kruskal–Wallis test).

Pale-green (PG) training

We successfully trained 26 butterflies to visit PG when the disks were presented on the black background. In the control test with a gray background, Gr1, 24 of them selected PG, the training color, from other colors in both tests 1 and 2 (P<0.05, Tukey's HSD test; Fig. 5A,B, white bars). For test 1, we used 20 of these butterflies, ten of which were tested with the B background and the other ten were tested with the MY background. The remaining four individuals were tested with both B and MY backgrounds in test 2.

When the same set of color disks was presented on the B background, the PG-trained butterflies visited not only PG but also BG in both tests 1 and 2 (Fig. 5A, black bars). In test 1, they visited BG significantly more than PG (P<0.05, Tukey's HSD test), and the overall selection pattern on the B background was significantly different from that on the Gr1 background (χ^2=38.36, P<0.01). The selection pattern in test 2 was also significantly different from that in the control test (χ^2=14.15, P<0.05; Fig. 5B, black bars). Here they visited PG most frequently, but no statistical significance was detected in the preference between PG and BG. The visit to BG was significantly different between the control and test 2 (χ^2=6.23 P<0.05), whereas the difference between the visits to PG in the control and in test 2 was not significant.

When the same set of color disks was presented on the MY background to the PG-trained butterflies, the selection pattern was significantly different from that of the control (test 1; χ^2=28.54, P<0.001, test 2; χ^2=31.00, P<0.01; Fig. 5B). They selected SG most frequently in both test 1 (P<0.05 Tukey's HSD test) and test 2,

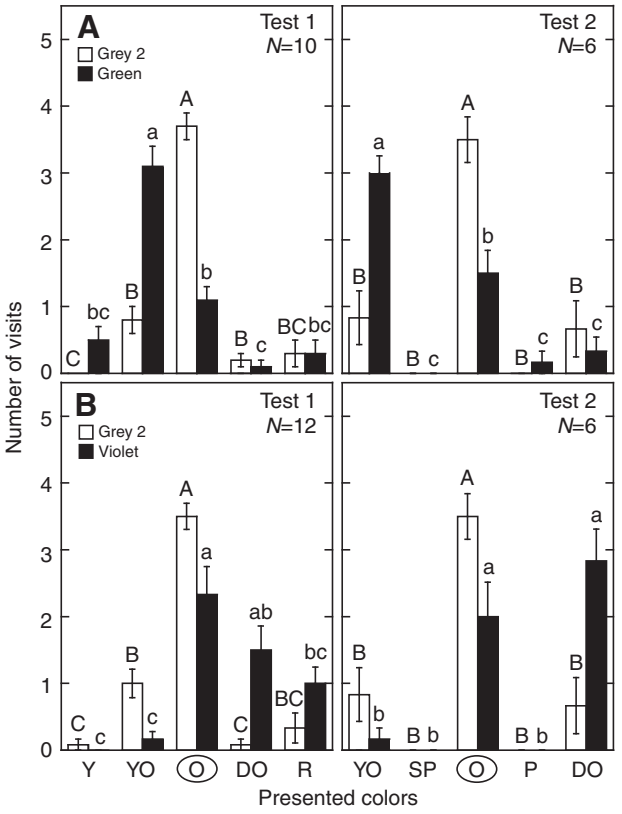

Fig. 6. Results of orange (O)-trained *Papilio*. Colors in test 1 were arranged linearly in the color space, whereas colors in test 2 were arranged radially around O, the training color (see Fig. 2C). (A) Effect of a green (G) background. (B) Effect of a violet (V) background. The choices were not random in each test. (A) Test 1: Gray (Gr2), H=35.64, P<0.0001; G, H=32.73, P<0.0001. Test 2: Gr2, H=21.54, P<0.01; G, H=22.66, P<0.01. (B) Test 1: Gr2, H=44.15, P<0.0001; V; H=29.75, P<0.0001. Test 2: Gr2, H=21.54, P<0.01; V, H=25.31, P<0.0001; Kruskal–Wallis test). Each bar shows the mean (±s.e.m.) number of visits. Letters above bars indicate statistical significance. Bars with different letters are significantly different (Tukey's HSD test, P<0.05). Capital letters are used for the results of the control test, and lowercase letters are used for the result of the color contrast tests.

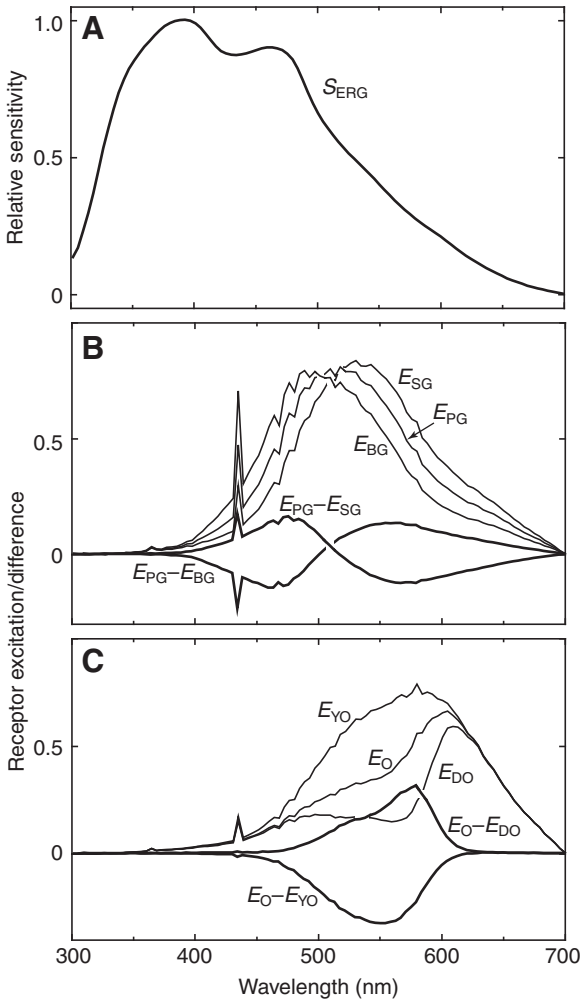

Fig. 7. Prediction of induced colors. (A) Summed spectral sensitivity of the ventral region of the *Papilio* retina measured by ERG recording [modified from Arikawa et al. (Arikawa et al., 1987)]. (B) Difference spectra of receptor excitations between pale green (PG) and the selected color, blue green (BG) with a blue (B) background, and spring green (SG) with a mustard yellow (MY) background. (C) Difference spectra of receptor excitations between orange (O) and the selected color, yellow orange (YO) with a green (G) background, and dark orange (DO) with a violet (V) background.

although statistical significance was not detected in test 2. When the background was changed from Gr1 to MY, the visits to SG and OG significantly increased (SG: χ^2=10.80, P<0.01; OG: χ^2=6.23, P<0.05), whereas the visits to PG significantly decreased (χ^2=12.60, P<0.001).

Orange (O) training

We trained 28 individuals successfully to O, and all of the O-trained butterflies visited O most frequently in the control test with background Gr2 (P<0.05, Tukey's HSD test; Fig. 6A,B). For test 1, we used 22 of them, 10 of which were tested with the G background and 12 were tested with the V background. The remaining six individuals were tested with both G and V backgrounds in test 2.

Color selection of the O-trained butterflies on the G background was different from that on the Gr2 background (test 1; χ^2=32.97, P<0.001, test 2; χ^2=13.81, P<0.01). When the background was changed to G, the O-trained butterflies visited YO most frequently in both tests 1 and 2 (P<0.05, Tukey's HSD test; Fig. 6A).

On the V background, the O-trained butterflies visited not only O but also DO and R in test 1 (Fig. 6B), which was different from that in the control test (χ^2=30.15, P<0.01). There were more visits to O and DO in test 1 than to each color in the control test (O; χ^2=5.79, P<0.05, DO; χ^2=16.01, P<0.01), whereas visits of YO decreased (χ^2=6.55, P<0.01). In test 2, they selected O and DO significantly more often (P<0.05, Tukey's HSD test; Fig. 6B), which was different from results of the control test (χ^2=13.17, p<0.01). When selecting between the V and Gr2 backgrounds in test 2, their visits to DO increased (χ^2=10.55, P<0.01), and those to O decreased (χ^2=4.31, P<0.05).

DISCUSSION

Simultaneous color contrast

Foraging *Papilio* butterflies changed their color preference when the background color was changed (Figs 5 and 6), but their preference was consistent even if the combination of presented colors was changed in tests 1 and 2. This result suggests that the perception of *Papilio* is strongly affected by the background, indicating that *Papilio* is capable of simultaneous color contrast. In fact, human

observers could also experience some degree of simultaneous color contrast when looking at the patterns we used here.

Induced color by background colors

The PG-trained butterflies selected the BG disk on B background (Fig. 5A), indicating that BG on B was similar to PG on black for *Papilio*, and the B background induced some color on the BG disk. To predict the induced color, we calculated the receptor excitation of color disk *i*, $E_i(\lambda)$, by:

$$E_i(\lambda) = S_{ERG}(\lambda)\, I(\lambda)\, R_i(\lambda), \quad (3)$$

where $S_{ERG}(\lambda)$ is the summed spectral sensitivity of the ventral region of the *Papilio* eye determined by electroretinographic (ERG) recording (Arikawa et al., 1987) (Fig. 7A), $I(\lambda)$ is the irradiation spectrum (Fig. 1A), and $R_i(\lambda)$ is the reflectance spectrum of paper *i* (Fig. 1). We then calculated difference spectra between the receptor excitations of the training and the selected colors. The difference spectrum between the E_{PG} and E_{BG} (E_{PG} minus E_{BG}) has a profile similar to the reflectance spectrum of the yellow paper (Fig. 1C), suggesting that the B background induced yellow (Fig. 7B). We also calculated the difference spectrum between the E_{PG} and E_{SG}. SG was selected on a MY background by the PG-trained butterflies. That spectrum (E_{PG} minus E_{SG}) has a broad peak at around 480 nm, which is similar to that of the blue paper (Fig. 1D), indicating that MY induced blue on SG, as expected (Fig. 7B).

Another set of induced colors is evident from the experiments using the O-trained butterflies. They selected YO on the G background and DO on a V background (Fig. 6). We calculated the difference spectra between E_O and E_{YO} and between E_O and E_{DO} (Fig. 7C). The former (E_O minus E_{DO}) appears to be similar to the reflectance spectrum of the green paper (Fig. 1D), whereas the latter (E_O minus E_{YO}) has a broad depression between 500 and 600 nm, similar to the reflectance spectrum of the violet paper (Fig. 1D).

These sets of colors, yellow/blue and green/violet, are probably so-called complementary colors for *Papilio*. However, complementary colors are defined as those that produce white when mixed, so it is necessary to verify this point in order to conclude that they are in fact complementary. By definition, there are infinite numbers of complementary color pairs in any color vision system. *Papilio* color vision is tetrachromatic, covering the wavelength region from UV to red (Koshitaka et al., 2008), similar to that of goldfish (Neumeyer, 1992; Dörr and Neumeyer, 1997). The behavioral experiments of simultaneous color contrast in both *Papilio* and goldfish did not involve the UV wavelength region, and how UV light is involved in the color induction system is still an open question.

Effect of brightness

The butterflies trained to a certain color disk on the black background correctly selected the training color on the gray background. Changing the background from black to gray decreases the brightness contrast between the disk and background; but the change in brightness contrast did not affect the selection behavior of *Papilio*.

It could be argued that *Papilio* used brightness difference of disks themselves as a cue; in fact, foraging hawkmoths use the brightness of their target as a cue in situations devoid of any chromatic difference (Kelber, 2005). In order to exclude that possibility, one must measure brightness increment thresholds first and then test butterflies using colors whose brightness values vary less than the measured detection thresholds. Although we did not test this very systematically, we did present five or seven color disks with brightness values that were somewhat variable (Fig. 4). The butterflies apparently selected both brighter and dimmer disks depending upon the color but not the brightness, suggesting that butterfly selection was most likely color dependent.

Possible neuronal mechanism

Simultaneous color contrast has been assumed to share the neuronal mechanism underlying color constancy (Hurlbert and Wolf, 2004; Vanleeuwen et al., 2007) which involves spatial integration of chromatic information. Physiological as well as anatomical evidence indicates that the horizontal cells in the goldfish retina play a crucial role in chromatic spatial integration, primarily because these horizontal cells are electrically coupled and also because they provide a strong feedback signal to the cone photoreceptors. This feedback mechanism leads to an increase in synaptic gain and mediates chromatic integration (Kamermans et al., 1998).

The neuronal mechanism underlying color contrast is still unknown in insects. In the case of *Papilio*, the spatial integration of chromatic information may begin at the level of the first visual ganglion, the lamina. Most photoreceptors with various spectral sensitivities extend into the lamina and make synaptic contact with axon collaterals of other photoreceptor cells and second-order visual interneurons, forming characteristic neuronal circuits (Takemura and Arikawa, 2006). The spectral types of interconnected photoreceptors are variable – that is, photoreceptors of the same spectral sensitivity are connected in some cases, and those of different spectral sensitivities are connected in other cases. Some photoreceptors even extend their collaterals into neighboring cartridges and make synaptic contacts with photoreceptors originating from neighboring ommatidia. The physiological properties of these photoreceptor connections may provide important insight into how the *Papilio* visual system integrates spatial chromatic information.

LIST OF ABBREVIATIONS

AB	aqua blue
B	blue
BG	blue green
DO	dark orange
FG	forest green
G	green
GB	grayish blue
Gr	gray
LG	lime green
MY	mustard yellow
O	orange
OG	olive green
P	pink
PG	pale green
R	red
SG	spring green
SP	salmon pink
V	violet
Y	yellow
YG	yellow green
YO	yellow orange

We thank D. G. Stavenga, D. C. Osorio, and C. McLaughlin for critical reading of the manuscript. We also thank Y. Takeuchi for helping us perform statistical analyses. This work was supported by research grants from the JSPS and from the Hayama Center for Advanced Studies of Sokendai to M.K. and K.A.

REFERENCES

Arikawa, K. (2003). Spectral organization of the eye of a butterfly *Papilio*. *J. Comp. Physiol. A* **189**, 791-800.
Arikawa, K., Inokuma, K. and Eguchi, E. (1987). Pentachromatic visual system in a butterfly. *Naturwissenschaften* **74**, 297-298.
Dörr, S. and Neumeyer, C. (1997). Simultaneous color contrast in goldfish: a quantitative study. *Vision Res.* **37**, 1581-1593.
Frisch, K. V. (1914). Der Farbensinn und Formensinn der Biene. *Zool. J. Physiol.* **37**, 1-238.

Hurlbert, A. and Wolf, K. (2004). Color contrast: a contributory mechanism to color constancy. *Prog. Brain Res.* **144**, 147-160.
Kamermans, M., Kraaij, D. A. and Spekreijse, H. (1998). The cone/horizontal cell network: a possible site for color constancy. *Visual Neurosci.* **15**, 787-797.
Kelber, A. (2005). Alternative use of chromatic and achromatic cues in a hawkmoth. *Proc. R. Soc. Lond., B, Biol. Sci.* **272**, 2143-2147.
Kelber, A. and Pfaff, M. (1999). True colour vision in the orchard butterfly, *Papilio aegeus. Naturwissenschaften* **86**, 221-224.
Kelber, A., Balkenius, A. and Warrant, E. J. (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature* **419**, 922-925.
Kinoshita, M. and Arikawa, K. (2000). Colour constancy of the swallowtail butterfly, *Papilio xuthus. J. Exp. Biol.* **203**, 3521-3530.
Kinoshita, M., Shimada, N. and Arikawa, K. (1999). Colour vision of the foraging swallowtail butterfly *Papilio xuthus. J. Exp. Biol.* **202**, 95-102.
Koshitaka, H., Kinoshita, M. and Arikawa, K. (2004). Action spectrm of foraging behavior of the Japanese yellow swallowtail butterfly, *Papilio xuthus. Acta Biol. Hung.* **55**, 71-79.
Koshitaka, H., Kinoshita, M., Vorobyef, M. and Arikawa, K. (2008). Tetrachromacy in a butterfly that has eight varieties of spectral receptors. *Proc. R. Soc. Lond., B, Biol. Sci.* **275**, 947-954.
Menzel, R. and Backhaus, W. (1989). Color vision in honey bees: phenomena and physiological mechanisms. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 281-297. Tokyo: Springer-Verlag.
Neumeyer, C. (1980). Simultaneous color contrast in the honeybee. *J. Comp. Physiol. A* **139**, 165-176.
Neumeyer, C. (1992). Tetrachromatic color vision in goldfish-evidence from color mixture experiments. *J. Comp. Physiol. A* **171**, 639-649.
Neumeyer, C., Dorr, S., Fritsch, J. and Kardelky, C. (2002). Colour constancy in goldfish and man: influence of surround size and lightness. *Perception* **31**, 171-187.
Takemura, S. Y. and Arikawa, K. (2006). Ommatidial type-specific interphotoreceptor connections in the lamina of the swallowtail butterfly, *Papilio xuthus. J. Comp. Neurol.* **494**, 663-672.
Vanleeuwen, M. T., Joselevitch, C., Fahrenfort, I. and Kamermans, M. (2007). The contribution of the outer retina to color constancy: a general model for color constancy synthesizd from primate and fish data. *Visual Neurosci.* **24**, 277-290.
Zaccardi, G., Kelber, A., Sison-Mangus, M. P. and Briscoe, A. D. (2006). Color discrimination in the red range with only one long-wavelength sensitive opsin. *J. Exp. Biol.* **209**, 1944-1955.